Chemotactic 5-Oxo-Eicosatetraenoic Acids Induce Oxygen Radical Production, Ca^{2+}-Mobilization, and Actin Reorganization in Human Eosinophils via a Pertussis Toxin-Sensitive G-Protein

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The arachidonic acid metabolites 5-oxo-[6*E*,8*Z*,11*Z*,14*Z*]-eicosatetraenoic acid (5oETE) and 5-oxo-15-hydroxy-[6*E*,8*Z*,11*Z*,13*E*]-eicosatetraenoic acid (5oHETE) are potent eosinophil chemotaxins. Here, the activation profile of 5-oxo-eicosanoids in eosinophils was further characterized and compared to other eosinophil activators such as complement fragment C5a (C5a), platelet-activating factor (PAF), interleukin-5 (IL-5), and phorbol ester (PMA). Flow cytometric studies revealed a rapid and transient actin polymerization upon stimulation by both 5-oxo-eicosanoids. Desensitization studies using actin polymerization as the parameter indicated cross-desensitization between the two 5-oxo-eicosanoids but revealed no interference with the response to other chemotaxins. Fluorescence measurements with Fura-2-labeled eosinophils in the presence of EGTA indicated Ca^{2+}-mobilization from intracellular stores by 5oETE and 5oHETE. Both 5-oxo-eicosanoids stimulated the production of reactive oxygen metabolites as demonstrated by lucigenin-dependent chemiluminescence, superoxide dismutase-inhibitable cytochrome C reduction, and flow cytometric dihydrorhodamine-123 analysis. At optimal concentrations the changes induced by 5-oxo-eicosanoids were comparable to those obtained by C5a and PAF, whereas IL-5 and PMA induced only a restricted pattern of cell responses. Cell responses elicited by 5-oxo-eicosanoids were inhibited by pertussis toxin, indicating coupling of the putative 5-oxo-eicosanoid-receptor to G-proteins. These results indicate that 5-oxo-eicosanoids are stong activators of eosinophils with comparable biologic activity to the eosinophil chemotaxins C5a and PAF. These findings point to a role of 5-oxo-eicosanoids in the pathogenesis of eosinophilic inflammation as chemotaxins as well as activators of pro-inflammatory activities. ***J Invest Dermatol 108:108–112, 1997***

Human eosinophils are considered major effector cells in several inflammatory conditions, e.g., parasitic infections, atopic diseases, and bullous dermatoses or vasculitis (Walker *et al*, 1991; Weller, 1992; Gleich *et al*, 1993; Bruijnzeel, 1994; Gounni *et al*, 1994). Recruitment of eosinophils into inflammatory tissue is presumably caused by different chemotactic agents. Well characterized eosinophil chemotaxins are the complement split product C5a (C5a), the chemokine RANTES, the phoshatidylcholine-derivative platelet-activating factor (PAF), and the arachidonic acid metabolite leukotriene B_4 (LTB_4) (Valone, 1980; Nagy *et al*, 1982; Wardlaw *et al*, 1986; Weber and Dahinden, 1995). In addition to migration, these substances stimulate eosinophil effector functions, such as the production of reactive oxygen metabolites, mediator release, and cell adhesion (Kapp *et al*, 1994). These proinflammatory activities may contribute to the toxic and tissue-destructive potency of eosinophils.

Activation of neutrophils by chemotaxins requires binding to membrane-spanning ligand-specific cell surface receptors (Baggiolini and Dahinden, 1994). At the intracellular site of the plasma membrane the receptors for chemotaxins such as C5a, PAF, LTB_4, and RANTES interact with pertussis toxin-sensitive heterotrimeric guanine nucleotide-binding proteins (G-proteins) (Gierschik *et al*, 1989; Baggiolini *et al*, 1993). Activated G-proteins dissociate into the α-guanosine triphosphate subunit and free $\beta\gamma$-dimers, which activate phospholipase C (Champs *et al*, 1992). This enzyme cleaves phosphatidylinositol (4,5)-bisphosphate into diacylglycerol and inositol trisphosphate (Berridge and Irvine, 1989). Diacylglycerol activates protein kinase C, and inositol trisphosphate mobilizes Ca^{2+} from intracellular stores (Berridge and Irvine, 1989). In addition, G-proteins are involved in the control of the actin cytoskeleton (Stossel, 1989). This later event occurs independently of activation of the phospholipase C. Actin reorganization is probably controlled by tight interactions between phospholipids and actin- binding proteins (Stossel, 1989). Intracellular Ca^{2+}-

Manuscript received April 4, 1996; revised August 2, 1996; accepted for publication August 15, 1996.

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Abbreviations: 5oETE, 5-oxo-[6*E*,8*Z*,11*Z*,14*Z*]-eicosatetraenoic acid; 5oHETE, 5-oxo-15-hydroxy-[6*E*,8*Z*,11*Z*,13*E*]-eicosatetraenoic acid; C5a, complement fragment C5a; LTB_4, leukotriene B_4; PAF, platelet-activating factor; G-proteins, heterotrimeric guanine nucleotide-binding proteins.

transients, protein kinase C, and actin reorganization in concert regulate leukocyte cell response such as the production of reactive oxygen species (Baggiolini *et al*, 1993).

Recently, 5-oxo-eicosanoids, i.e., 5-oxo-[6*E*,8*Z*,11*Z*,14*Z*]-eicosatetraenoic acid (5oETE) and 5-oxo-15-hydroxy-[6*E*,8*Z*,11*Z*,13*E*]-eicosatetraenoic acid (5oHETE) were identified as potent chemotaxins for human eosinophils (Powell *et al*, 1995; Schwenk and Schröder, 1995). Desensitization experiments suggested that 5oETE and 5oHETE bind to the same receptors, which are distinct from other chemotaxin receptors (Powell *et al*, 1992; Schwenk *et al*, 1992). In this study, the signal pathways and cell effector functions elicited by 5-oxo-eicosanoids in human eosinophils were characterized and compared to the effect induced by other eosinophil activators.

MATERIALS AND METHODS

Materials Recombinant human C5a (C5a), α-phosphatidylcholine-β-acetyl-γ-*O*-hexadecyl (PAF), leukotriene B_4 (LTB_4), phorbol 12-myristate 13-acetate (PMA), lysophosphatidylcholine, lucigenin, and ficoll paque were obtained from Sigma (Deisenhofen, Germany); recombinant human interleukin-5 (IL-5) from Peprotech (London, England); *N*-(7-nitrobenz-2-oxa-1,3-diazol-4yl)-phallacidin (NBD-phallacidin) and dihydrorhodamine-123 from Becton Dickinson (Heidelberg, Germany); {1-[2-(5-carboxy-oxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, pentaacetoxymethyl ester} (Fura-2) from Calbiochem (La Jolla, Ca); the monoclonal antibody against CD16 (BW 209/2) used for purification of eosinophil granulocytes was obtained by Dr. R. Kurrle (Behring-Werke, Marburg, Germany); immunomagnetic beads (Dynabeads M-450) were purchased from Dianova (Hamburg, Germany); 5oETE and 5oHETE were synthesized as described (Schwenk *et al*, 1992; Schwenk and Schröder, 1995); pertussis toxin was a kind gift of Prof. Dr. K. Aktories (Freiburg, Germany).

Isolation of Eosinophils Human eosinophil granulocytes were isolated from heparin-anticoagulated blood of healthy volunteers by ficoll separation and negative selection with anti-CD16 antibody-coated Dynabeads as described (Czech *et al*, 1993). The purity of the isolated eosinophils was ≥ 96% as judged by Pappenheim stain.

Actin Polymerization The filamentous actin content was analyzed by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) with NBD-phallacidin staining (Norgauer *et al*, 1989). Briefly, aliquots of cell suspension (5×10^5 eosinophils per ml) were withdrawn at the indicated time intervals from a stirred and 37°C temperated sample compartment. Equal volumes of cells (100 μl) were fixed in a 7.4% formaldehyde buffer and mixed with the staining cocktail containing 7.4% formaldehyde, 0.33 μM NBD-phallacidin, and 1 mg lysophosphatidylcholine per ml. The fluorescence intensity was measured.

Intracellular Ca^{2+}-Measurements Intracellular free Ca^{2+} was measured in Fura-2–labeled cells with an Aminco Bowman series 2 fluorospectrometer (SLM Instruments, Urbana, IL) (Norgauer *et al*, 1993). Eosinophils (2×10^6 cells per ml) were incubated with 1 μmol Fura-2 for 15 min at 37°C in Ca^{2+}- and Mg^{2+}-free buffer. Cells were washed twice and finally resuspended in a buffer containing 1.5 mmol $CaCl_2$ and $MgCl_2$. The fluorescence traces after stimulation were followed fluorospectrometrically, and the ratio between 340 nm and 380 nm was calculated.

Lucigenin-Dependent Chemiluminescence Eosinophils were resuspended to a density of 2.5×10^5 cells per ml in HEPES buffer containing 200 μM lucigenin. Aliquots (200 μl) were placed into polystyrene luminescence tubes (Lumacuvette/Abimed, Düsseldorf, Germany). Measurements were performed in triplicate at 37°C. The reaction over a 60-min time period after addition of stimuli to the cells were followed and expressed as intensity integral counts (Kapp *et al*, 1994).

Production of Hydrogen Peroxide Production of intracellular hydrogen peroxide was quantified by flow cytometry as described (Elsner *et al*, 1994). Briefly, eosinophils were incubated with 1 μM dihydrorhodamine-123 at 37°C for 5 min. The fluorescence intensity after stimulation was quantified by flow cytometry.

Superoxide Anion Production The production of superoxide anions was measured as superoxide dismutase-inhibitable reduction of cytochrome C at 550 nm (Ultrospec III, Pharmacia, Freiburg, Germany) (Norgauer *et al*, 1988).

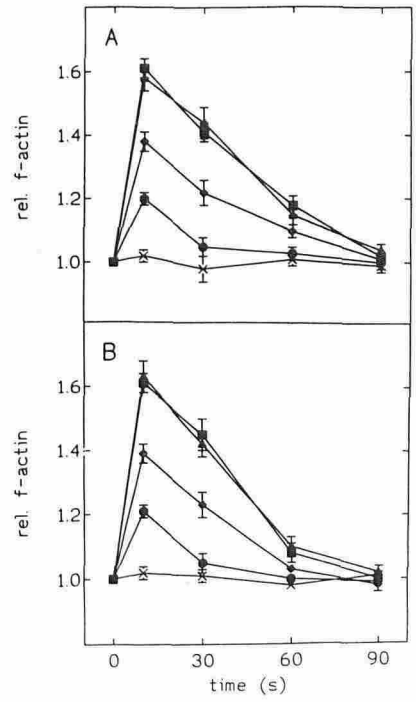

Figure 1. Effect of 5oETE and 5oHETE on actin-polymerization in eosinophils. Cells were stimulated with 1000 nM (■) 100 nM (▲), 10 nM (♦), 1 nM (●), and vehicle (X) of 5oETE *(A)* or 5oHETE *(B)*. The relative f-actin content was determined at the indicated time points by flow cytometry. Data are means ± SEM (n = 5).

RESULTS

Actin Response Induced by 5oETE and 5oHETE The influence of 5-oxo-eicosanoids on the actin network was analyzed by flow cytometry. Addition of 5oETE and 5oHETE to eosinophils caused a rapid and transient polymerization of actin molecules **(Fig 1*A*,*B*)**. There was an increase of the f-actin content of about 50% within 10 s after addition of the stimuli. The time required for return to baseline values depended on the concentration of the added stimuli. At maximal concentration the responses were finished 90 s after exposure of eosinophils to 5-oxo-eicosanoids. Half-maximal and maximal effects after stimulation with both agents were observed at 10 nM and 100 nM, respectively.

For analyzing the desensitization of the 5-oxo-eicosanoid-induced actin response, the eosinophils were exposed to a maximal concentration of the indicated first stimulus, which was either a 5-oxo-eicosanoid or another eosinophil chemotaxin such as C5a, PAF, or LTB_4. These agents stimulated actin responses with similar time courses and extensions as 5-oxo-eicosanoids. After reaching the blank value, 5oETE or 5oHETE was added 5 min after the first stimulus. As depicted in **Table I**, both 5-oxo-eicosanoids revealed

Table I. Desensitization of the 5-oxo-Eicosanoid–Induced Actin Response[a]

First Stimulus		5oETE[b]	5oHETE[b]
None		1.65 ± 0.05	1.69 ± 0.08
5oETE	(100 nM)	1.06 ± 0.05	1.07 ± 0.04
5oHETE	(100 nM)	1.04 ± 0.07	1.04 ± 0.05
PAF	(100 nM)	1.59 ± 0.06	1.60 ± 0.08
C5a	(100 nM)	1.56 ± 0.08	1.58 ± 0.06
LTB_4	(1000 nM)	1.58 ± 0.06	1.59 ± 0.08

[a] Eosinophils were exposed for 5 min to the indicated concentration of the first stimulus. Subsequently, a second stimulation for 10 s with 100 nM 5oETE or 5oHETE was performed. The relative f-actin content in comparison to control cells is given.
[b] Data are means ± SEM (n = 3).

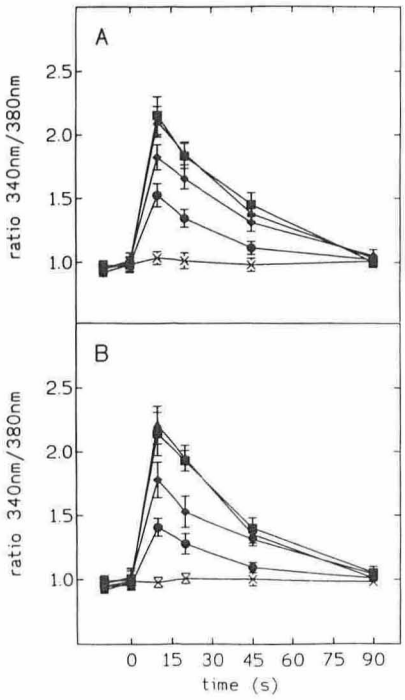

Figure 2. Time course of 5oETE- and 5oHETE-stimulated intracellular Ca^{2+}-transients in eosinophils. Cells were labeled with Fura-2 and stimulated with 1000 nM (■), 100 nM (▲), 10 nM (♦), 1 nM (●), and vehicle (X) of 5oETE *(A)* or 5oHETE *(B)*. Data are means ± SEM (n = 3).

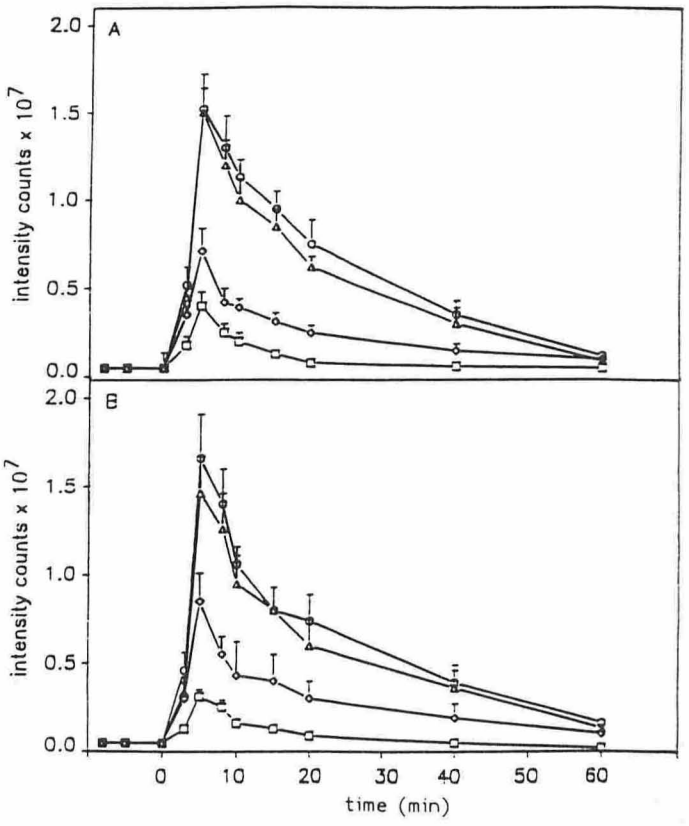

Figure 3. Dose dependency of the 5oETE- and 5oHETE-induced lucigenin-dependent chemiluminescence response in eosinophils. Cells were stimulated with 1000 nM,(■) 100 nM (○), 10 nM (△), and 1 nM (◇) of 5oETE *(A)* or 5oHETE *(B)*. Data are means ± SEM (n = 5).

homologous desensitization. In addition, 5oETE was capable of desensitizing responses to 5oHETE and *vice versa*. None of the other chemotaxins tested interfered with the 5-oxo-eicosanoid response.

Mobilization of Intracellular Ca^{2+} by 5oETE and 5oHETE Intracellular Ca^{2+}-transients were followed by fluorospectrometry in Fura-2-labeled eosinophils. Both 5oETE and 5oHETE induced rapid and concentration-dependent increases in intracellular Ca^{2+} with equal potency **(Fig 2*A,B*)**. Addition of EGTA prior to stimulation allows increases in intracellular Ca^{2+} to be distinguished from those caused by mobilization from intracellular stores and those caused by influx across the plasma membrane (Norgauer *et al*, 1993). As demonstrated in **Table II** EGTA did not affect stimulated Ca^{2+}-transients, indicating mobilization from intracellular stores exclusively.

Activation of the Respiratory Burst by 5-oxo-eicosanoids The activation of the respiratory burst by 5-oxo-eicosanoids in eosinophils was followed by lucigenin-dependent chemiluminescence. Both 5-oxo-eicosanoids induced the production of reactive oxygen species in a concentration-dependent manner **(Fig 3*A,B*)**. These continuous measurements indicated a rapid induction with a maximum after 5 min. Intracellular production of hydrogen peroxide in eosinophils was analyzed by flow cytometry **(Table III)**. Again, both 5-oxo-eicosanoids increased the intracellular fluorescence of dihydrorhodamine-123-labeled eosinophils, indicating activation of the respiratory burst. The superoxide anion production after stimulation with 5oETE and 5oHETE was analyzed by superoxide dismutase-inhibitable cytochrome C reduction **(Table IV)**. Optimal concentrations of 5oETE and 5oHETE generated about 5 nmol superoxide anion/10^6 eosinophils within 30 min. Similar amounts of superoxide anion were also found after longer stimulation periods (up to 60 min, data not shown).

Comparison of the Activation Profiles of Different Eosinophil Stimuli The activation profile of 5-oxo-eicosanoids was compared to the responses provoked by other eosinophil activators such as C5a, PAF, IL-5, and phorbol ester. Similar to 5-oxo-

Table II. Influence of Ethyleneglycol-bis(β-Aminoethyl Ether)-N,N,N,N′-Tetraacetic Acid (EGTA) on 5-oxo-Eicosanoid-Induced Intracellular Ca^{2+}-Transients[a]

Stimulus	EGTA	Ratio[b]
None	−	0.95 ± 0.08
None	+	0.93 ± 0.07
5oETE	−	2.21 ± 0.14
5oETE	+	2.15 ± 0.17
5oHETE	−	2.28 ± 0.21
5oHETE	+	2.09 ± 0.13

[a] Twenty seconds before stimulation with 5-oxo-eicosanoids, 4 mM EGTA or control medium was added to Fura-2–labeled eosinophils. Cells were stimulated with and without 100 nM 5oETE or 5oHETE. The ratio was quantified 10 s after addition of the stimuli in the presence or absence of EGTA in the extracellular medium.
[b] Data are means ± SEM (n = 3).

Table III. Induction of Hydrogen Peroxide Production by 5-oxo-Eicosanoids[a]

Stimulus	Fluorescence[b]
Control	195 ± 15
5oETE	344 ± 38
5oHETE	315 ± 50
PMA	678 ± 87

[a] Eosinophils were loaded with dihydrorhodamine-123 and intracellular production of hydrogen peroxide after stimulation for 30 min without or with 100 nM 5oETE, 100 nM 5oHETE, or 10 ng/ml PMA was quantified by flow cytometry.
[b] Data are given as means ± SEM (n = 4).

Table IV. Influence of 5-oxo-Eicosanoids on Superoxide Anion Production[a]

Stimulus	Superoxide Anions nmol/10^6 Cells[b]
Control	0.7 ± 0.2
5oETE	5.7 ± 1.0
5oHETE	5.6 ± 0.8
C5a	6.2 ± 0.5
PAF	6.1 ± 0.4
PMA	14.5 ± 2.1

[a] Eosinophils were stimulated without or with 100 nM 5oETE, 100 nM 5oHETE, 100 nM C5a, 100 nM PAF for 30 min, and 10 ng PMA per ml for 60 min. Superoxide anion production was quantified by superoxide dismutase–inhibitable cytochrome C reduction.

[b] Data are means ± SEM (n = 3).

eicosanoids, C5a and PAF at optimal concentrations induced intracellular Ca^{2+}-transients, stimulated actin reorganization, and triggered the respiratory burst **(Table V)**. In contrast to 5-oxo-eicosanoids, IL-5 and PMA at concentrations up to 1000 U per ml or 50 ng per ml had no effect on intracellular Ca^{2+}-transients and actin response but induced the production of oxygen metabolites. The continuous measurements of lucigenin-dependent chemiluminescene revealed a different time course of oxygen metabolite production induced by 5-oxo-eicosanoids, IL-5, and PMA **(Fig 4)**, the latter two agents requiring a longer lag phase than 5-oxo-eicosanoids.

Pertussis Toxin Inhibition of 5-oxo-Eicosanoid–Induced Cell Responses. Pertussis toxin blocks cell activation induced by G_i-protein–coupled receptors (Gierschik *et al*, 1989). Pretreatment of eosinophils with pertussis toxin completely inhibited 5-oxo-eicosanoid-induced Ca^{2+}-transients, actin reorganization, and chemiluminescence response **(Table VI)**. To prove the metabolic activity of eosinophils after pertussis toxin treatment, the chemiluminescence response with PMA was followed. Toxin treatment did not influence the phorbol ester–triggered response.

DISCUSSION

Well defined chemotaxins for eosinophils are C5a, PAF, and LTB_4 (Valone, 1980; Norgauer *et al*, 1982; Wardlaw *et al*, 1986; Weber and Dahinden, 1995). Recently, two novel potent granulocyte-derived chemotaxins for eosinophils, the arachidonic acid metabolites 5oETE and 5oHETE, have been identified (Powell *et al*, 1995; Schwenk and Schröder, 1995). To improve our understanding of the biologic activities of 5-oxo-eicosanoids, we analyzed various intracellular signal mechanisms and cell effector functions in eosinophils. As could be expected for a chemotactic agent, we have shown here that 5-oxo-eicosanoids induced a transient reorganization of the actin network. The precise regulation mechanisms for the actin response is not fully understood; however, it is believed to involve interaction of phospholipids with actin-binding proteins (Stossel, 1989). This study demonstrated mobilization of Ca^{2+} from intracellular stores by 5-oxo-eicosanoids. Similar responses have been reported after stimulation of eosinophils with the chemotaxins C5a and PAF (Kroegel *et al*, 1989; Kernen *et al*, 1991; Elsner *et al*, 1994). Release of sequestered Ca^{2+} into the cytosol might be caused by soluble inositol trisphosphate generated by activation of phospholipase C (Berridge and Irvine, 1989). This enzyme cleaves phosphatidylinositol bisphosphate into inositol trisphosphate and diacylglycerol, which is a potent activator of protein kinase C (Berridge and Irvine, 1989). Intracellular Ca^{2+}-transients and protein kinase C have been implicated in many biologic regulative mechanisms including the activation of the nicotinamide adenine dinculeotide oxidase (Baggiolini *et al*, 1993). Production of reactive oxygen metabolites by 5-oxo-eicosanoids was demonstrated here with several different methods. This finding could be of relevance for the pro-inflammatory activity of eosinophils in diseases with an eosinophilic infiltrate.

Cell biology studies and cloning of the cDNA of the C5a- and PAF-receptors revealed interaction of these chemotaxin receptors with G-proteins (Gerard *et al*, 1989; Ye *et al*, 1991). Here we have shown that all cell responses induced by 5-oxo-eicosanoids were inhibited by pertussis toxin. This inactivates heterotrimeric G_i-proteins by adenosine diphosphate ribosylation (Gierschik *et al*, 1989). This suggests coupling of the putative receptors for 5-oxo-eicosanoids to heterotrimeric G_i-proteins. The data presented here about cross-desensitization of the actin response between 5-oETE

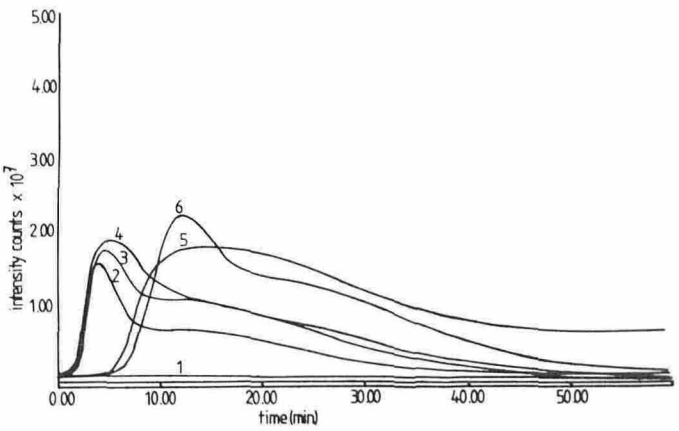

Figure 4. Time course of lucigenin-dependent chemiluminescene induced by different activators in eosinophils. The time course of the lucigenin-dependent chemiluminescence response in eosinophils upon stimulation without (line 1) or with 100 nM C5a (line 2), 100 nM 5oETE (line 3), 100 nM PAF (line 4), 10 ng PMA per ml (line 5), and 100 U IL-5 per ml (line 6) is shown. Representative data of one experiment are shown. The experiment was repeated five times with identical results.

Table V. Influence of Different Eosinophil Activators on Actin Response, Ca^{2+}-Transients, and Lucigenin-Dependent Chemiluminescence[a]

Stimulus	f-Actin[b]	Ca^{2+}-Transients[b]	Chemiluminescence[b]
Control	1.00 ± 0.00	0.87 ± 0.04	16 ± 4
5oETE	1.52 ± 0.07	2.15 ± 0.21	391 ± 41
5oHETE	1.49 ± 0.04	2.07 ± 0.14	352 ± 53
C5a	1.53 ± 0.06	2.42 ± 0.09	342 ± 58
PAF	1.47 ± 0.04	2.29 ± 0.08	454 ± 49
IL-5	1.04 ± 0.03	0.91 ± 0.03	522 ± 62
PMA	1.02 ± 0.02	0.86 ± 0.05	608 ± 78

[a] Eosinophils were stimulated without or with 100 nM 5oETE, 100 nM 5 oHETE, 100 nM C5a, 100 nM PAF, 100 U IL-5 per ml, or 10 ng phorbol ester (PMA) per ml. The relative actin content and the ratio for the intracellular Ca^{2+}-measurements were taken after 10 s. Chemiluminescence response is given as integral (counts × 10^6) after 60 min.

[b] Data are mean ± SEM (n = 3).

Table VI. Effect of Pertussis Toxin on 5-oxo-Eicosanoid–Induced Cell Activation[a]

Stimulus	Toxin	f-Actin[b]	Ca^{2+}-Transients[b]	Chemiluminescence[b]
None	–	1.00 ± 0.00	0.87 ± 0.09	15 ± 3
None	+	0.98 ± 0.03	0.86 ± 0.08	14 ± 4
5oETE	–	1.53 ± 0.06	2.03 ± 0.15	376 ± 56
5oETE	+	1.10 ± 0.05	1.02 ± 0.09	22 ± 6
5oHETE	–	1.47 ± 0.07	1.98 ± 0.21	349 ± 61
5oHETE	+	1.11 ± 0.03	0.98 ± 0.07	24 ± 7
PMA	–	1.04 ± 0.06	0.86 ± 0.07	624 ± 102
PMA	+	1.03 ± 0.05	0.83 ± 0.04	602 ± 87

[a] Eosinophils were incubated with and without 10 μg pertussis toxin per ml for 2 h. Thereafter the cells were stimulated with and without 100 nM 5oETE, 100 nM 5oHETE, or 10 ng phorbol ester (PMA) per ml. The relative f-actin content and the ratio for the intracellular Ca^{2+}-transients were quantified 10 s after stimulation. The integral intensity counts of lucigenin-dependent chemiluminescence after 60 min is given.

[b] Data are mean ± SEM (n = 3).

and 5oHETE, as well as previously described cross-desensitization of induced Ca^{2+}-transients, would suggest common receptors for both 5-oxo-eicosanoids in eosinophils (Schwenk and Schröder, 1995). Because there was no cross-desensitization between 5-oxo-eicosanoids and other eosinophil chemotaxins such as PAF, C5a, and LTB_4, these putative receptors should be distinct from known chemotaxin receptors.

In contrast to previous reports in neutrophils (Powell *et al*, 1992), the concentration response studies shown here revealed 5oETE and 5oHETE as equipotent activators. It must be considered that α,β-unsaturated oxo-components spontaneously equilibrate with corresponding enol-forms. Conjugated cis-double bounds, as in both 5-oxo-eicosanoids, spontaneously form the corresponding trans-products, which have weaker chemotactic activity for eosinophils than the corresponding Δ_6-cis forms (Schwenk and Schröder, 1995). Therefore, formation of trans-products could account for the discrepancy.

Although 5-oxo-eicosanoids at least partially activated similar signal pathways in neutrophils, a pronounced quantitative difference in their capacity to induce the respiratory burst in eosinophils and neutrophils was noticed (O'Flaherty *et al*, 1993; Norgauer *et al*, 1996). The molecular basis of the difference in the activation capacity by 5-oxo-eicosanoids in these two types of granulocytes is not known. One explanation would be different numbers of receptors. On the other hand, the effectiveness of receptor–G-protein coupling depends on the composition of G-proteins (Kleuss *et al*, 1993). These signal molecules are composed of three different subunits, the guanosine triphosphate–binding α-subunits and $\beta\gamma$-dimers (Hepler and Gilman, 1992). Multiple subtypes of the different subunits are known (Hepler and Gilman, 1992). The expression pattern and expression level of the different G-protein subtypes in eosinophils and neutrophils are currently not known. Various expression patterns or levels of these proteins could therefore explain the different responsiveness of eosinophils and neutrophils.

The different activation profile of 5-oxo-eicosanoids in neutrophils and eosinophils point to a functional role of these agents in the pathogenesis of eosinophilic inflammation. At present, the instability of the reactive 5-oxo-eicosanoids limits the proof of these agents *in vivo* by skin diseases. Recent studies, however, revealed synthesis of 5-oxo-eicosanoids in normal and transformed keratinozytes (M. Barbisch and J. Norgauer, unpublished observations). Moreover, injections of 5oETE into rabbits provoked a massive eosinphilic infiltrate in the dermis (J. Norgauer and Schraufstatter, unpublished observations). Therefore, one can speculate that 5-oxo-eicosanoids might play a central role in the pathogenesis of inflammatory skin diseases with an eosinophilic infiltrate such as parasitic infections, atopic diseases, bullous dermatoses, and vasculitis.

The results of this study indicate that 5-oxo-eicosanoids in eosinophils induce actin polymerization and intracellular Ca^{2+}-mobilization via pertussis toxin–sensitive G-proteins. In addition, they are strong activators of the respiratory burst. These findings point to a role of 5-oxo-eicosanoids in the pathogenesis of eosinophilic inflammation as chemotaxins as well as activators of proinflammatory activities.

This work was supported by grants of the Deutsche Forschungsgemeinschaft. Material described herein is an essential part of the thesis of M. Barbisch.

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